UNIVERSITY OF GONDAR

College of Natural and Computational Science
Department of Biology, Applied Microbiology

THE EFFECT OF BACILLUS SPECIESS AND CYANOBACTERIA ON THE GROWTH OF PEPPER (*Capsicum annuum L.*), RICE (*Oryza sativa L.*) AND TOMATO (*Lycopersicon esculentum* L.) SEEDLINGS.

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A Thesis Submitted to the Department of Biology University of Gondar in the Partial Fulfillment of the Requirement for the Degree of MSC in Applied Microbiology

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Abbreviation

ANOVA	Analysis of variance
Bt	*Bacillus thuringiensis*
GMO	Genetically Modified Organisms
GRAS	Generally Regarded As Safe
ISR	Induction of Systemic Resistance
LSD	List Significance Difference
N_2O	Nitrous oxide
PGPR	Plant Growth Promoting Rhizobacteria
USFDA	United state Food and Drug Administration
SPSS	Statical Package for Social Science
TSI	Triple Sugar Iron

Abstract

Excessive use of agrochemical was one mechanism of increase crop production and which leads to environmental damage. Microorganisms are important for agriculture in order to promote the circulation of plant nutrients and reduce the need for chemical fertilizers. Today researchers are able to repeatedly use them successfully in field experiments to increase growth and yields of some crop and vegetables in different nations; however data on the effect of cyanobacteria on the growth of rice, tomato and pepper seedlings is inadequate. The aim of this study was to isolate and evaluate the effect of cyanobacteria on the growth of rice, tomato and pepper seedlings with deferent cultivars. The study designs were randomized, purposeful laboratory based experiment. Sediments sample used to isolate Bacillus and soil sample used to isolates Cyanobacteria were collected randomly from Lake Tana and from Gondar Teacher Education Collage respectively. The seeds of Peppers and Tomato were collected from Adet Agriculture Research Center and Rice seeds from Woreta Rice Research Center. The seedling of pepper, rice and tomato were prepared in University of Gondar, Biology department field experiment station. Pot experiments in laboratory were conducted to evaluate the effect of Cyanobacteria and Bacillus on Rice, Pepper and Tomatoes seedling. Five Bacillus and two Cyanobacteria species were isolated and identified from Lake Tana and from Lake Tana and from Gondar Teacher Education Collage respectively. All five Bacillus and tow Cyanobacterial isolates showed positive effects on the growth of pepper, rice and tomato seedlings as compared to the control, however their effectiveness varies from isolate to isolate and also from cultivar to cultivar. Among all Cyanobacteria isolate (C1) was considered as having more plant growth promoting ability in all cultivars of tomato Melkesalsa (70%, 70.4%), kochero (75%, 76%) and Miya (92.5%, 79%) shoot length and root length respectively relative to other. In pepper plant cv. Endiam42 from all isolates C1 provide more growth promotion with efficacy (80.73%,66. 72%) shoot length and root length respectively and in cv. Markofana B4 was showed higher efficacacy(81.5%, 73.86%) shoot length and root length respectively relative to other. In Rice cv. X-jigna and cv. Edget C2 showed higher growth promotion efficacy (37.93%, 50.53%) and (50.07%, 52.9%) respectively. Thus, microorganisms could be considered as one of the possible biofertilizer that leads to increase the health and vigor of seedlings. PGPR tests are recommended to be done on fields how much increasing the yield of the crop and there should be demonstrated to the end users who are having Agricultural importance.

Key Words: Bacillus, Cyanobacteria, Pepper, Rice, Tomato

1. INTRODUCTION

The issue of soil productivity has become a global concern as soil fertility is diminishing gradually for many reasons including soil erosion, nutrient mining, accumulation of salts and other toxic elements. Intensification of agriculture emphasizes heavy use of chemical fertilizers, which leads to adverse environmental effects. Nitrous oxide (N_2O) is an example of chemical pollutant produced by excessive use of nitrogen fertilizer and is a major source of greenhouse gases causing global warming (Butterbach *et al.*, 2013). Micro-organisms are important for agriculture in order to promote the circulation of plant nutrients and reduce the need for chemical fertilizers. Plant growth promoting rhizobacteria (PGPR) are able to exert a beneficial effect upon plant growth (Lucy *et al*., 2004).

Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria which have the ability to colonize the roots and promotes plant growth through direct action or through biological control of plant diseases (Kloepper and Schroth, 1978). They are associated with many plant species and are commonly present in varied environments. Strains having PGPR (Plant growth promoting rhizobacteria) activity, belonging to genera *Azoarcus, Azospirillum, Azotobacter, Arthrobacter, Bacillus, Clostridium, Enterobacter, Gluconacetobacter, Pseudomonas,* and *Serratia*. Among these, species Pseudomonas and Bacillus are the most extensively studied. These bacteria competitively colonize the roots of plant and can act as bio fertilizers and/or antagonists (bio pesticides) or simultaneously both (Kloepper and Schroth, 1978).

To full fill the world agricultural vision, crops production needs to be ready with disease resistance, salt tolerance, drought tolerance, heavy metal stress tolerance, and better nutritional value. To fulfill the above preferred crop properties, one possibility is to use soil microorganisms including bacteria, fungi, algae and cyanobacteria that increase the nutrient uptake capacity and water use efficiency or having plant growth promoting ability (Armada *et al.*, 2014). PGPR (Plant Growth Promoting Rhizobacteria) play great role in agriculture productivities improvement through enhance plant health or promote plant growth rate without environmental contamination (Calvo *et al.*, 2014).

The current agricultural practice (use of conventional chemical farming methods), which is significantly increased crop production, was once regarded as a kind of agriculture revolutions, which would solve all problems relating to producing sufficient food for the ever growing world population. However, this belief was later over shadowed by the emergence associated with the heavy use of agrochemicals in intensive farming systems. Conventional farming methods are generally associated with degradation of the environment. Among other things, soil degradation is one of the most serious problems, which affect crop production. Increasing prices of agrochemicals especially nitrogen, often leaves the marginal farmers with low profits (Calvo *et al.*, 2014). Unavailability of those agrochemicals, especially in the developing countries is often a serious constraint for the farmers in their attempt to increase crop production (Calvo *et al.*, 2014).

A positive impact of PGPR (plant growth-promoting rhizobacteria) on initial growth of pepper has been described previously (Garcia *et al.,* 2004; Joo *et al.*, 2005; Russo, 2006), none of the previous studies were done under environmental and cultural conditions found in Ethiopia. Thus, the utility of PGPR (plant growth-promoting rhizobacteria) as inoculant bio fertilizers in Ethiopia is not well understood. The inhibitory effect of *Bacillus* on plant pathogenic fungi has been frequently reported in laboratory, greenhouse, and field studies (Pusey and Wilson, 1984). The present study is, therefore, initiated in North Gondar, Ethiopia in the seedling growth promotion of Bacillus on Rice, Tomato and Pepper.

The recognition of plant growth-promoting rhizobacteria (PGPR), a group of beneficial plant bacteria, as potentially useful for stimulating plant growth and increasing crop yields has evolved over the past several years to where today researchers are able to repeatedly use them successfully in field experiments. Increased growth and yields of potato, sugar beet, radish and sweet potato (Farzana *et al*., 2009) have been reported. Data on the effect of cyanobacteria for the growth of Rice, Tomato and Pepper seedlings is inadequate. Therefore, the aim of this study is to isolate and evaluate the effect of cyanobacteria for the growth of Rice, Tomato and Pepper seedlings. This study was help seedlings to escape preliminary growth problems such as disease, nutrient computations or to escape preliminary death of rice, tomato and pepper seedlings to give sufficient increase in yield.

2. LITRAETURE REVIEW

2.1. What are Plant Growth Promoting Rhizobacteria (PGPR)?

Plant growth promoting Rhizobacteria (PGPR) are group of bacteria that can be found in the rhizosphere. The term "plant growth promoting Rhizobacteria" refers to bacteria that colonize the roots of plants (rhizosphere) that enhance plant growth. The rhizosphere is the soil environment where the plant root is available and is a zone of maximum microbial activity resulting in a confined nutrient pool in which essential macro and micronutrients are extracted. The microbial population present in the rhizosphere is relatively different from that of its surroundings due to the presence of root exudates that function as a source of nutrients for microbial growth (Burdman *et al*., 2000).

Bacteria, fungi, acticomycetes, protozoa, and algae are the microorganisms colonizing rhizosphere. However, bacteria are the most abundant microbes present in the rhizosphere. The term "plant growth promoting Rhizobacteria (PGPR)" was introduced by Kloepper and Schroth (Kloepper and Schroth, 1978) paving the way for greater discoveries on PGPR. PGPR are not only associated with the root to exert beneficial effects on plant development but also have positive effects on controlling phytopathogenic microorganisms (Kloepper *et al*., 1980). Therefore, PGPR serve as one of the active ingredients in biofertilizer formulation.

Based on the interactions with plants, PGPR can be divided into symbiotic bacteria, whereby they live inside plants and exchange metabolites with them directly, and free-living Rhizobacteria, which live outside plant cells (Gray and Smith, 2005). The working mechanisms of PGPR can also be divided into direct and indirect ones. The direct mechanisms are bio fertilization, stimulation of root growth, rhizoremediation, and plant stress control. On the other hand, the mechanism of biological control by which Rhizobacteria are involved as plant growth promotion indirectly is by reducing the impact of diseases, which include antibiosis, induction of systemic resistance, and competition for nutrients and niches(Egamberdieva and Lugtenberg, 2014).

2.2. Importance of PGPR on current Agriculture system

PGPR play an important role in enhancing plant growth through a wide variety of mechanisms. The mode of action of PGPR in promoting plant growth includes (i) abiotic stress tolerance in plants; (ii) nutrient fixation for easy uptake by plant; (iii) plant growth regulators; (iv) the production of siderophores; (v) the production of volatile organic compounds; and (vi) the production of protection enzyme such as chitinase, glucanase, and ACC-deaminase for the prevention of plant diseases (García-Fraile *et al*., 2015).

2.3. Bacillus as plant growth promoting bacteria

Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria which have the ability to colonize the roots and either promote plant growth through direct action or via biological control of plant diseases (Kloepper and Schroth, 1978). Strains of *Bacillus* and Paine bacillus play important role in enhancing plant growth through a wide variety of mechanisms.

The inhibitory effect of *Bacillus subtilis* on plant pathogenic fungi has been frequently reported in laboratory, greenhouse, and field studies .*B. subtilis* is able to synthesize more than 60 different types of antibiotics, mainly in polypeptides, many of which possess antifungal effects and belong to the iturin family (Compant *et al.*, 2005). Besides the anti-fungal effects, some compounds produced by *B. subtilis* may also act as plant growth promoters (Compant *et al.*, 2005). *Bacillus subtilis SY1* was used in the experiments to determine its bio control effect on some pathogenic fungi in vegetable soil (Compant *et al.*, 2005). Various species of *Bacillus* are dominant in soil and are known to secrete antimicrobials and siderophores. In addition, they are also important microbial producers of bio surfactants such as rhamnolipis. These can cause lysis of zoospores of soil borne plant pathogenic fungi such as *Pythium*, *Phytophthora* and *Plasmopara* by interacting with and disrupting their plasma membrane (Stanghellini and Miller, 1997). Consequently, Bacillus can facilitate a control of damping-off especially in vegetable nurseries where the disease is often prevalent by destroying zoospores of this phyto pathogen. Interestingly, protection against diseases by rhizobacteria also involves quorum sensing in rhizosphere (Gray and Smith , 2005, Sharma *et al*., 2003) and induction of systemic resistance(ISR) wherein lipo polysaccharides have been reported to act as signal molecules(Pathak *et al*., 2004).

Leclère *et al*. (2005) revealed that lipo poly sac ride(LPs) are important determinants of bio control activity, when he found that over production of myco subtilin, which is a member of iturin family; by *B. subtilis* strain BBG100 had significant antagonistic properties against phyto pathogenic fungi, such as *Pythium aphanidermatum* on tomato seedlings.

2.4. Interest in Bacillus as bio pesticides

Bacterial products represent the majority of the microorganism-based bio pesticides but fungal bio control agents were also developed as efficient products (Shoresh *et al.,* 2010). Among the bacterial bio control agents, *Bacillus thuringiensis* accounts for more than 70% of total sales. This bacterium is essentially used for insect pest control and is the origin of the gene used in insect resistant ‘‘Bt GMO crops (Shoresh *et al.,* 2010).

The genus *Bacillus* encompasses a large genetic biodiversity. *Bacilli* are present in an extremely large palette of environments ranging from sea water to soil, and are even found in extreme environments like hot springs (Hoch *et al*., 1993). This bacterium could be one of the major sources of potential microbial bio pesticides because it retains several valuable traits (Ongena and Jacques, 2008). Firstly, Bacilli, such as *B. subtilis*, are well-studied organisms that can facilitate their rational use. Secondly, the US Food and Drug Administration (USFDA) has granted the "generally regarded as safe" (GRAS) status to *Bacillus subtilis* which is thus recognized non-pathogenic (Harwood and Wipat, 1996).This is of course essential regarding its application as a bio pesticide. Thirdly, Bacilli have the capacity to produce spores (Piggot and Hilbert, 2004) which are extremely resistant dormancy forms capable to withstand high temperatures, unfavorable pH, lack of nutrients or water, etc. They are produced by the bacteria when environmental conditions are unfavorable which probably helps these microorganisms to survive in the phyto sphere. The phenomenon can also be exploited in industrial production as sporulation can be induced at the end of cultures (Monteiro *et al.,* 2005). This greatly facilitates post-culture conditioning as bacterial suspensions can be converted to easy to handle powder formulations without the impressive bacterial mortality observed with non-sporulating bacteria (Lolloo *et al*., 2010). Shelf life of bio pesticides based on sporulated bacteria is generally longer and require less storage precaution compared to other products containing living organisms.

Bacilli are also relatively easy to produce industrially as they are not particularly exigent regarding nutritional sources. Beside its spore forming ability, *B. subtilis* possess several characteristics that enhance its survival in the rhizosphere and thus its effectiveness as a bio pesticide (Rosas-Garcia, 2009). This bacterium known to live in aerobic environments can also behave as facultative anaerobe surviving and evolving under low oxygen concentration (Nakano and Hulett, 1997). This is a real advantage in the rhizosphereas oxygen availability may fluctuate during time and is generally low. Additionally, *B.subtilis* is a motile bacterium that readily moves towards and on the root surface which facilitates colonization of new ecological niches. Another reason for the high interest in *Bacilli* is the diversity of their modes of action. They can display almost all the mechanisms of bio control and bio-stimulation/fertilization mentioned here below and above. Moreover, one strain may often acts through several mechanisms. This enables these bacteria to be effective in many conditions (variety of pathogens, plants, environmental conditions) as one mechanism may act instead of another.

2.5. Mechanisms involved in bio control of plant diseases by *Bacillus*

By taking benefits from the nutrients constantly released from roots or leaves of growing plants, beneficial bacterial strains efficiently colonize leaf surfaces and root systems and their surrounding soil layer. In turn, they beneficially influence the plant by protecting it from infection by plant pathogens via three main mechanisms: competition for ecological niche/substrate, production of inhibitory allele chemicals, and induction of systemic resistance in host plants. It should be noted that none of these mechanisms described above are necessarily mutually exclusive, and frequently several modes of action are exhibited by a single bio control agent. In the next sections, we mainly consider beneficial microbes introduced in soil but the same principles and mechanisms apply for isolates used to combat foliar diseases (Alabouvette *et al.,* 2006).

2.5.1. Competition for niche and nutrients

Competition for resources such as nutrients and oxygen occurs generally in soil among soil inhabiting organisms. For bio control purpose, it occurs when the antagonist directly competes against pathogens for these resources. Root inhabiting micro organisms competes for suitable

sites at the root surfaces. Competition for nutrients, especially for carbon, is assumed to be responsible for the well-known phenomenon of fungi stasis, characterizing the inhibition of fungal spore germination in soil (Alabouvette *et al.,* 2006). Given the relatively low abundance of substrates in the rhizosphere, the efficiency of nutrient uptake and catabolism by bacteria is a key factor in competitiveness. Competition for trace elements, such as iron, copper, zinc, manganese etc., also occurs in soils. For example, iron is an essential growth element for all living organisms and the scarcity of its bio-available form in soil habitats results in a furious competition (Loper and Henkels, 1997). Siderophores, low molecular weight compounds with high iron affinity, are produced by some microorganisms (and also by most bio control agents) to solubilize and competitively acquire ferric ion under iron-limiting conditions, thereby making iron unavailable to other soil microorganisms which cannot grow for lack of it (Loper and Henkels,1997). Suppression of soil borne plant pathogens through competition for niche has been demonstrated in some instances for some beneficial bacteria such as *Pseudomonas* (Haas and Défago, 2005).

2.5.2. Direct inhibition of phyto pathogens

Members of multiple *Bacillus* species such as *B. amyloliquefaciens, B. subtilis, B. cereus, B.licheniformis, B. megaterium, B. mycoides,* and *B. pumilus* are known as very efficient producers of antibiotic molecules (Stein, 2005). *Bacillus subtilis* has an average of 4-5% of its genome devoted to antibiotic synthesis and has the potential to produce more than two dozen structurally diverse antimicrobial compounds (Stein, 2005). In the case of soil borne diseases, iturin A produced by *B. subtilis* RB14 was involved in the control of damping-off of tomato (a seedling disease) caused by *Rhizoctonia solani* (Asaka and Shoda,1996).

The ability of bacteria to parasitize and degrade spores or hyphae of pathogens through the production of various cell-wall degrading enzymes has also been suggested (Whipps, 2001). As examples, isolates related to *Bacillus ehimensis* (Hoster *et al.,* 2005) produce chitin-degrading enzymes while *Bacillus subtilis* AF1displays some fungi toxicity through the secretion of *N*-acetyl glucose aminidase and glucanase (Manjula and Podile, 2005).

2.6. Importance and general characteristics of cyanobacteria

Cyanobacteria are oxygenic, photosynthetic prokaryotic organisms that are distributed worldwide and can inhabit a wide range of habitats including freshwater, marine and terrestrial environments (Tripathi *et al*., 2007). Cyanobacteria are cosmopolitan prokaryotic microorganisms that can be found in a wide array of habitats, from marine to fresh waters, from soil to rocks, dwelling in temperate and extreme climates. Due to their low nutrient requirements and their high adaptability to environmental conditions, some have long been known to grow at high latitudes, characteristic of what was defined as "astonishing" ,at temperatures exceeding 40 °C (which is the highest temperature tolerated by diatoms living in hot springs and in hyper saline environments . Although 35 °C is the optimal temperature for growth, some Cyanobacterial species were observed at temperatures as high as 85 °C (Fogg, 1956).

The utilization of Cyanobacteria in agriculture has the following economic benefits (reduced input cost), nutrient cycling, N_2-fixation, bioavailability of phosphorus, water storage and movement, environmental protection and prevention of pollution and land degradation especially through reducing the use of agro-chemicals, and recycling of nutrients and restoration of soil fertility through reclamation(Burja *et al.,* 2001).

2.7. Plant growth promoting mechanism of Cyanobacteria

Application of Cyanobacteria as a bio fertilizer serves a number of purposes, most importantly the enrichment of the soil and plants with different compounds. There are indications that application of some Cyanobacteria strains is able to fix atmospheric nitrogen and enrich the soil with this crucial microelement for plants. This process, as a means of nitrogen fertilization, is being used in rice and wheat cultivation and can be beneficial in ecological agriculture (Burja *et al.,* 2001). In addition, it is thought that Cyanobacteria and green algae can produce beneficial growth regulators and active compounds (classified as secondary metabolites) that inhibit the growth of pathogenic bacteria and fungi and can increase growth and development of some plant species. Some papers suggest that Cyanobacterial activity improves soil structure and porosity by secretion of polysaccharides and mucilage (Burja *et al.,* 2001).

2.7.1. Nitrogen Fixation by Cyanobacteria

Nitrogen is an essential constituent of proteins, nucleic acids, chlorophylls, enzymes, and other physiological substances in green plants. Nitrogen is the macronutrient that is required in high amounts by plants, and its availability in the soil may change substantially in relatively short time intervals (Cameron and Haynes, 1986). For rapid growth of all plants, nitrogen is probably the most common limiting factor. Hence, an adequate supply of nitrogen in agriculture is very important (Chuang, 1984). Cyanobacterial N2 fixation and cyanobacteria could contribute to the natural fertility of the soils through nitrogen-fixation in their heterocysts and/or vegetative cells(Chuang, 1984).

2.7.2. Production of Growth-Promoting Substances

Cyanobacteria excrete a great number of substances that influence plant growth and development. These microorganisms have been reported to benefit plants by producing growth promoting regulators (the nature of which is said to resemble gibberellins and auxins), vitamins, amino acids, polypeptides, antibacterial and antifungal substances that exertphyto pathogen bio control and polymers, especially exo polysaccharides, that improve soil structure and exo enzyme activity (Zaccaro, 2000). Moustafa and Omar (1990) reported that inoculation of tomatoes with a mixture inoculum of *Azospirillum lipoferum* and cyanobacteria, formally called blue-green algae (a mixture of different cyanobacteria strains) and/or cyanobacteria alone as bio fertilizer led to increase significantly as improved the quality of tomato fruits. Also, Kotb *et al*. (1990) showed that inoculation with *Azorpirillum* and /or algae gave significant positive differences for fresh weight of tomato fruits and plants dry weight when compared to the control plants without inoculation.

Zeenat and Sharma (1990) reported that the inoculation of tomato with cyanobacteria in presence of reduced chemical nitrogen fertilizer (75 % N) improved tomato plants growth and increased significantly the yield compared to control treatment without inoculation. They suggested that cyanobacteria secreted considerable amounts of growth–promoting substances into the surrounding medium, thereby increasing the growth and yield of tomato**.**

2.7.3. Transformation of Soil Phosphorus

Phosphorus is the second major plant nutrient after nitrogen in terms of quantitative requirements for crop plants. The problem of P management in soil is highly intricate, as the applied phosphate through fertilizers is often fixed and becomes unavailable to the crops. In organic matter rich soils, P availability is due to excretions of enzymes or acidic metabolites produced by microorganisms including cyanobacteria (Rogers *et al*,. 1991).

2.7.4. Improvement in Soil Physical Properties

Cyanobacteria are known to excrete extracellularly a number of compounds like polysaccharides, Peptides and lipids during their growth in soil .These compounds diffuse around soil particles, glue and hold them together in the form of micro aggregates. Besides these compounds, polysaccharides are made of fibers, which can also entangle clay particles and form clusters. These clusters or micro aggregates, in turn, grow and take the shape of macro aggregates and subsequently of larger soil aggregates. The interwoven nature of growing algal filaments may also help in binding the soil particles along with the organic C added through algal biomass. The importance of these compounds in soil-aggregate formation or soil stabilization has been indicated by many workers (Rogers *et al*,. 1991).

2.8. Statement of the Problems

Increasing prices of agrochemicals especially nitrogen, often leaves the marginal farmers with low profits. Uncertain availability of those agrochemicals, especially in the developing countries such as Ethiopia, is often a serious constraint for the farmers in their attempt to increase crop production.

Currently, among the most important factors limiting production of different crops are soil-borne plant pathogens. By this reason, different methods have been used to control these pathogens. Cultural practices and chemical control using synthetic fungicides are the most used control methods; however, use of some of these synthetic products has caused various problems due to environmental pollution, with consequences such as toxicity to humans, as well as resistance of certain pathogens to these fungicides (Sid *et al*., 2003). Modern agriculture is intensively depended on use of agrochemicals (herbicides, insecticides, Fungicides etc). This is practiced to

increase the global food production by killing crop pests but at the same time, it has started polluting the environment.

Nitrogen, an essential macronutrient limiting agricultural productivity is the largest and most costly input in agriculture. Though atmospheric and dissolved dinitrogen (N_2) in soil and water is in plenty however, due to its chemical inertia most of the plants (except those in symbiotic associations with N_2 fixers) are unable to utilize it (Prasanna *et al*., 2013).

Phosphorus is the second major plant nutrient after nitrogen in terms of quantitative requirements for crop plants. Phosphorus deficiency is widespread and phosphorus fertilizers are almost universally required to maintain crop production because when it is added to soil in the form of phosphatic fertilizers, only a small part of phosphorus is utilized by plants and the rest is converted into insoluble fixed forms. The problem of P management in soil is highly intricate, as the applied phosphate through fertilizers is often fixed and becomes unavailable to the crops (Rogers *et al*., 1991).

Tomato is one of the most important vegetables because of its health benefits and phytochemical properties. Because of its low calorie and absence of cholesterol, it is one of the recommendations of diets needing low cholesterol. They are quite rich in many important nutrients and vitamins which include phosphorus and potassium and also vitamins B and C. They are also very important against common cancers like breast and prostate cancer (Babalola and Glick, 2012). As important as tomato is nutritionally and in being an important cash crop for smallholders and medium-scale commercial farmers in Africa, soil-borne pathogens inflicts a lot of diseases and infections on it (Babalola and Glick, 2012). Such diseases include Bacterial wilt, root knot nematodes disease, early blight, late blight and Fusarium wilt. Fusarium wilt is a devastating disease of tomato and causes a lot of loss to farmers worldwide(Babalola and Glick, 2012).

2.9. Significance of the Study

Currently the incidence of pesticide resistant organisms is increasing. As a result there is an urgent need for new controlling methods which are effective against current pesticide resistant pathogens. Therefore, this study give a clue about choice of the potential organisms used as bio control for pesticide resistant plant pathogens.

The study helps to assess the effect of Bacillus and cyanobacteria on the growth of tomato, rice and pepper seedling. Furthermore, it provides base line information for further studies on the effect of bacillus and cyanobacteria on the growth of tomato, rice and pepper seedling.

Modern agriculture is intensively depended on use of agrochemicals (herbicides, insecticides, Fungicides etc). This is practiced to increase the global food production by killing crop pests but at the same time, it has started polluting the environment. Therefore, this study give a clue about organic farming to overcome the problem of environmental pollution.

Nitrogen, an essential macronutrient limiting agricultural productivity is the largest and most costly input in agriculture. Though atmospheric and dissolved dinitrogen (N_2) in soil and water is in plenty however, due to its chemical inertia most of the plants are unable to utilize it. Therefore, this study give a clue about the choice, isolation and utilization of the potential organisms which have the ability to fixes atmospheric nitrogen.

2.10. Objectives of the study

2.10.1. General objective

The general objective of this research is to evaluate the effect of bacillus species and cyanobacteria on the growth of tomato, rice and pepper seedlings.

2.10.2. Specific objectives

- ✓ To isolate, characterize and screen *Bacillus* spp from Lake Tana sediments for promoting seedling growth of pepper, rice and tomato.
- ✓ To isolate and screen Cyanobacteria from Gondar area for promoting seedling growth of pepper, rice and tomato.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The soil sample used to isolate cyanobacteria was collected from around Gondar located in the north western Ethiopia at a latitude of 12^0 36' N, and longitude of 37^0 28' E with an elevation of 2133 meter above sea level. It is 747 Km far from Addis Ababa in the North West direction. According to the Central Statistical Agency of Ethiopian (2010) report, it has 20^oC average temperature and 1800mm rain fall and the warmest average maximum temperature is 29°c (84°F) in March and May. The coolest average minimum temperature is 10°c (50°F) in January and December. The mean relative humidity for an average is recorded as 55.7% and on a monthly basis it ranges from 40% in January and February to 79% in July.

Samples of sediments used to isolates bacillus were collected from Lake Tana, Amhara regional state, Ethiopia and from reserved samples previously collected by Muluken. The sampling area is located at a latitude of 12 (12° 0' 0 N) and a longitude of 37.33 (37° 19' 60 E). The lake has total surface area 3,600.00 km^2 with volume of 28.00 km^3 and has an average elevation of 1911 meters above the sea level. Lake Tana is the largest lake in Ethiopia and is the source of the Blue Nile. Lake Tana is formed by a volcanic blockage that reversed the previously north-flowing Blue Nile and created one of Africa's greatest waterfalls, (LAKE NET, 2003).

3.2. Study design

The study designs were randomized, purposeful and laboratory based experiment.

3.3. Sampling and sample collection method

Sediment samples used to isolate Bacillus species were collected randomly from Lake Tana and the soil sample used to isolate Cyanobacteria were collected randomly from Teacher Education Collage of Gondar.

The soil sample used for cyanobacteria isolation was collected from three different sites of Teacher Education Collage of Gondar by sterilized spatula and transferred to sterilized polyethylene bag from the upper part of the selected sites through a zigzag format in depth of 20 to 30 cm and 500 grams from each site. For Bacillus isolation sediments samples were collected by

sterilized spatula and transferred to sterilize polyethylene bag from three different sites of Lake Tana 500 grams from each site. All samples were labeled and transported to Microbiology Laboratory, Department of Biology University of Gondar and stored at 4 °C for further studies.

Seeds of Pepper and Tomato were collected from Adet Agricultural Research Center and Rice seed from Woreta International Rice Research Center. The seedlings of Pepper, Rice and Tomato were prepared at University of Gondar, biology department field experiment station.

3.4. Pretreatment Methods for Selective Isolation of *Bacillus*

Physical pretreatment methods were applied on the sediment samples used to isolate Bacillus species to facilitate isolation of *Bacillus* species. The sediment samples were air dried through covering by sterilize polyethylene bag, heat treated (80^0c) aseptically as the pre treatment of sediments by drying and heating stimulates the isolation of *Bacillus* species by eliminating the most unwanted fungal elements and Gram negative bacteria(Zhang, 2005).

3.5. Screening of Samples for *Bacillus*

Bacillus species were isolated by serial dilution method from sediments. One gram of each sediment sample was taken and diluted in 9ml of distilled water and shaken well using vortex. From the stock culture solution, 1 ml suspension was transferred aseptically to the 1st tube (10^{-2}), and mixed well then 1 ml of suspension was transferred into 2nd tube (10^{-3}), and mixed well. Similarly, dilutions up to 10^{-5} were made (serial dilution technique). Finally 0.1 ml of suspension from 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} culture tube was spread on nutrient agar medium plates aseptically. For each sample three plates were used and incubated at 37^0C for 24 hour. Representative colonies of all morphological types were picked separately at random and sub cultured on nutrient agar media by streaking plate method for purification. The purified cultures were maintained on nutrient agar slant at 4°C for further use (Benson, 2002).

3.6. Characterization of *Bacillus*

The *Bacillus* isolates were characterized by morphological, biochemical and physiological methods (Rampelotto, 2010).

3.6.1. Colony Characterization

The selected isolates were streaked aseptically on nutrient agar media and incubated at 37^{o}C for 24 to 48 hrs. Colony characteristics were observed as described by (Manga and oyeleke, 2008).

3.6.2. Gram's staining

Colonies that were grown on nutrient agar was Gram stained in accordance with standard Gram staining procedure described by Todar *et al*. (2005).

3.6.3. Biochemical and Physiological Characterization

The isolates were subjected to a series of biochemical and physiological testes which include indol, Catalase, MR-VP, TSI, citrate utilization, anaerobic growth, hydrolysis of starch, gelatin, casein and urea, growth at different NaCl concentrations and temperature (Manga and Oyeleke, 2008).

3.7. Cyanobacteria isolation and characterization

Soil sample was collected and transferred under aseptic conditions to the laboratory and stored at 4°C in the laboratory. One gram of each soil sample was taken and diluted in 9ml of distilled water and shaken well using vortex. From the stock culture solution, 1 ml suspension was transferred aseptically to the 1st tube (10^{-2}), and mixed well then 1 ml of suspension was transferred into 2nd tube (10^{-3}), and mixed well. Finally 0.1 ml of suspension from 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} culture tube was spread on BG11 medium (Rippka *et al.,* 1979) containing cyclohexamide (100 µg/ml) for the isolation of *Cyanobacteria* aseptically. For each sample three plates were used and incubated at 25 ± 2°C for 7 days. Representative colonies of all morphological types were picked separately at random and sub cultured on BG11 media by streaking plate method for purification. This process was repeated three times and pure culture was obtained. After purification the strains were characterized morphologically. Finally the purified cultures were maintained on nutrient agar slant at 4°C for further use.

3.8. Inoculum Preparation

A single colony of Bacillus was transferred to 100-ml flasks containing 25 ml of nutrient broth and grown aerobically in the flasks at 37 for 2°C 4 hours. The bacterial suspension was then

diluted in sterile distilled water and the prepared suspensions were used to inoculate in to prepared seedling.

A single filament of Cyanobacteria was transferred to 100-ml flasks containing 25 ml of BG11 and grown aerobically in the flasks at 25 °C for 7 days. The Cyanobacteria suspension was then diluted in sterile distilled water and the seedlings were reniesed to the prepared suspension and wait for some seconds and transplant to prepared pots which filled with 3kg soil.

3.9. Plant growth experiments

Pot experiments in laboratory were conducted to evaluate the effect of Cyanobacteria and Bacillus on Rice, Pepper and Tomato seedlings. Seeds of Pepper and Tomato were collected from Adet Agricultural Center and, Rice seed from Woreta Rice Research Center were sown on the prepared soil and watered regularly up to 45 days. The seedlings were dipped in the bacterial spore suspensions and Cyanobacterial filament suspension or in distilled water (control) for some minutes immediately before transferring to prepared pot. In addition, for each treatment, 100 ml of a spore suspension at the same concentration as the one used to treat seedlings were poured on the soil surface surrounding each seedling in every transplanting three replication for each treatment. Pots were observed regularly and watered up to 45 days after inoculation 600 ml waters per days for tomato and pepper and 800 ml for rice in two rounds (evening and morning) to one pot.

3.10 .Data collected from pot experiments

Pepper, Rice and Tomatoes seedlings were treated with Bacillus and Cyanobacteria species and transplant to pots with three replications. After 45 days, the seedlings were uprooted and their vegetative parameters including stem and root fresh and dry weights, root length, and stem length were measured.

3.11. Efficacy test

Efficacy test was applied to determine the effectiveness of each isolates on each cultivar by the following formula

Efficacy= treated-control/treated**x**100% (Stirling *et al.,*1998*)*

3.12. Data analysis

The collected data were subjected to different statistics such as percentages, means and frequencies. The data were also summarize in the form of tables, graphs and charts to facilitate comparison, to get the required information in less time and also statistical analysis were done by using SPSS 20 software version. A p-value of less than 0.05 was considered to indicate statistical significance.

4. RESULTS AND DISCUSSION

4.1. RESULTS

4.1.1. Isolation *Bacillus* Species

Five bacterial colonies with different growth characteristics were successfully isolated from sediments samples obtained from Lake Tana. They were designated as B1, B2, B3, B4 and B5 where "B" stands for *Bacillus*. The isolates were identified based on morphological observation, microscopic observation and biochemical characterization (Table1and 2). Bergey's manual of determinative of bacteriology was used as a reference to identify the isolates.

4.1.2. Morphological characteristics of *Bacillus* isolates

The beginning observation concerning the colony morphology of the isolated bacterial strains is presented in Table 1.

4.1.3. Physiological and Biochemical Characteristics of *Bacillus* isolates

Physiological and biochemical characteristics result indicates that all isolates produced Catalase, *Bacillus* isolates B1, B2 and B5 had the capacity to produce H_2S, gas and acid and *Bacillus* isolates B2,B3 and B4 utilize citrate as a carbon source.

Hydrolytic enzyme production ability of the isolates was observed. It revealed that all the isolated species except B5 were able to hydrolyze casein; B1 and B4 were able to hydrolyze starch; Gelatin hydrolysis was observed in all species except B2.

Growth response of *Bacillus* isolates at different temperature (20°C, 30°C, 35°C, 37°C, 45°C and 55°C) revealed that they were able to survive at different temperatures and have optimal growth at 35°C and 37°C, where as B3 able to grow at 20°C. On the other hand B1 grow at 45°C and 55°C. All the isolates except B3 able to grow in 5% concentration of sodium chloride, where as only B3 were resistant to 10% sodium chloride.

According to Bergeys manual of determinative microbiology, the isolates were identified as B1 (*B. thermophiles),* B2 *(B.thuringiensis),* B3 **(***B.sphaericus),* B4 (*B.licheniformis),* and B5(*B.pumilus)* (Claus and Berkeley, 1986).

4.1.4. Isolation of *Cyanobacteria*

Two types of colonies were obtained and purified through strike plate method. Strains were designated as C1 and C2, where "C" stands for *Cyanobacteria*. The isolates were identified based on morphological observation and microscopic observation (Table 3). www.algaebase.org was used as a reference to identify the isolates. The isolates were identified as C1 *Anabaena* and C2 *Nostoc.*

4.1.5. Morphological characteristics of *Cyanobacteria* isolates

The beginning observation regarding the colony morphology of the isolated *Cyanobacterial* strains is presented in Table 3.

4.1.6. Effect of *Bacillus a*nd *Cyanobacteria* on the growth of Pepper, Rice and tomato seedlings

Pepper, Rice and tomatoes seedlings were treated with *Bacillus* and *Cyanobacteria* species and transplant to pots with three replications. After 45 days, the seedlings were uprooted and their vegetative parameters including stem and root fresh and dry weights, root length, and stem length were measured.

4.1.7. Effect of *Bacillus* and *Cyanobacteria* on the growth of Tomato seedlings

The effect of *Bacillus* and *Cyanobacteria* isolates on the growth of Tomato seedlings was investigated. All five bacillus isolates (B1, B2, B3, B4 and B5) and tow cyanobacteria isolates (C1 and C2) show positive effects on the seedling growth of all 3 Tomato cultivars Melke salsa (Table4)**,** Kochero (Table5), and Miya (Table6) in all growth parameters including stem and root fresh and dry weights, root length, and stem length, however deferent isolates were showed deference effects on deferent cultivars.

It was observed that treated seedlings of cv. Melkesalsa with B5 *Bacillus* isolate showed higher shoot length than in the other *Bacillus* strain and uninoculated control. From all treatment C2 was showed the highest growth promotion in cv. Melkesalsa as compared to all *Bacillus* isolates and C1 and non-treated control in all growth parameter. And also from all treatment B4 was showed lower growth promotion in cv. Melkesalsa.

Tomato cv. Melkesalsa seedlings treated with C1show highest shoot length (50.0 ±2.51cm), shoot fresh weight (16±0.88gs), shoot dray weights (14.5±1.20gs), root length (14±0.52cm), root

fresh weight (4.40±0.11gs) and root dray weight (2±0.06gs) as compared to control and other treatment and the minimum value were recorded on the seedlings treated with B4 in all growth parameter shoot length (25.6 ±3.75cm), shoot fresh weight (7.33±0.33gs), shoot dray weights (5.20±0.60gs), root length (8.0±0.57cm), root fresh weight (2.2±0.03gs) and root dray weight (0.76±0.08gs) with statically significance at $p<0.05$ level of confidence (Table 4).

Table 4: The effect *Bacillus* and *Cyanobacterial* isolates on the growth of tomato cv. Melke salsa seedlings.

Treatment	Above ground growth parameter			Belowground growth parameter		
	Shoot length in cm	Shoot fresh weights in g	Shoot dray weights in g	Root length in cm	Root fresh weights in g	Root dray weights in g
B1	33.23±2.3 b*	15.3±0.33a**	12.5±0.6 a**	12±0.57 a*	2.9±0.03 a*	1.06±0.07 b*
B2	27.76±3.6 b*	7.66±0.33 b*	6.0±0.17 b*	10.4±0.29 a*	2.6±0.17 b*	0.93±0.03 b*
B3	35.0±5.68 b*	11.33±2.6 a*	8.66±2.3 b*	12.9±1.8 a**	3.1±0.18 a*	1.40±0.23 a*
B4	25.6±3.75 b*	7.33±0.33 b*	5.20±0.6 b*	8.0±0.57 b*	2.2±0.03 b*	0.76±0.08 b*
B5	40.3±1.85 b*	12.1±1.46 a*	8.33±0.6 b*	10.6±0.88 a*	2.8±0.05 b*	0.98±0.0b*
C1	50.0±2.51 a**	16±0.88 a**	14.5±1.2 a**	14±0.52 a**	4.40±0.11a**	2±0.06 a**
C2	42.3±0.33 b*	8.6±0.33 b*	6.3±0.33 b*	10.33±1.2 a*	3.56±0.23 a*	1.79±0.11a**
Control	15.00±1.00	1.80±0.25	1.00±0.1	4.0±0.57	0.93±0.03	0.59±0.03
LSD at0.05	3.2	3	2.2	3.3	0.98	0.93

Note: Values are mean± Standard error of three replications, *indicates statistically significant at $p<0.05$ (significant deference with control), ** indicates statistically significant at $p<0.01$(highly significant deference with control), Means in each column followed by the same letter are not significantly different at $p<0.05$ according to Fisher'S LSD.

Figure 2 Tomato seedlings in pot experiments

It was observed that treated seedlings of Tomato cv. Kochero seedlings treated with C1show the highest shoot length (50.6 ±0.20cm), shoot fresh weight (21.3±0.33gs), shoot dray weights (17.5±0.20gs), root length (15±0.57cm), root fresh weight (4.40±0.05gs) and root dray weight (2.96±0.03gs) as compared to control and other treatment and the minimum values were recorded on the seedlings treated with B3 in all growth parameters shoot length(16.53±1.00cm), shoot fresh weight(6.93±0.17gs), shoot dray weight(5.03±0.53gs), root length(6.8±0.27cm), root fresh weight(2.2±0.05gs) and root dray weight(1.08±0.04gs) with statically significance at $p<0.5$ (Table5). Table 5 showed that among Bacillus species B1 show the maximum shoot length, shoot fresh weghite,shoot dray weghite, root length, root fresh weghit and root dray weghite in the seedlings of cv. Kochero.

Table 5: The effect Bacillus and Cyanobacterial isolates on the growth of tomato cv. Kochero seedlings

Treat	Above ground growth parameter			Below ground growth parameter		
	Shoot length in cm	Shoot fresh weights in g	Shoot dray weights in g	Root length in cm	Root fresh weights in g	Root dray weights in g
B1	34.86±3.1b*	10.86±0.4b*	8.33±0.76b*	12.0±0.0a**	3.0±0.06a*	1.2±0.08b*
B2	19.53±2.02b*	7.20±0.2b*	5.50±0.25c*	7.3±0.88b*	2.6±0.03b*	1.03±0.03b*
B3	16.53±1.00b*	6.93±0.17b*	5.03±0.53c*	6.8±0.27b*	2.2±0.05b*	1.08±0.04b*
B4	33.60±2.0 b*	10.06±0.28*	8.03±0.54b*	8.0±0.57b*	2.2±0.09b*	1.14±0.04b*
B5	30.30±0.17b*	6.63±0.27b*	5.03±0.43c*	6.7±0.14b*	2.0±0.03b*	1.02±0.03b*
C1	50.6±0.2 a**	21.3±0.33a**	17.5±0.2a**	15±0.57a**	4.4±0.05a**	2.96±0.03a**
C2	40.30±2.25a*	11.9±0.58b*	8.83±0.4b*	13±0.57a**	3.4±0.18a**	1.63±0.12b*
Cont	12.20±0.1	1.73±0.08	0.88±0.00	3.6±0.33	1.06±0.07	0.66±0.14
LSD at0.05	2.8	2.6	2.56	2.1	2	1.8

Note: Values are mean± Standard error of three replications, *indicates statistically significant at $p<0.05$ (significant deference with control),** indicates statistically significant at $p<0.01$(highly significant deference with control), Means in each column followed by the same letter are not significantly different at $p<0.05$ according to Fishr,sLSD

Figure3: Underground growth parameter of tomato (on the left treated and on the right untreated)

Tomato Cv. Miya seedlings treated with *Bacillus* and Cyanobacteria showed growth enhancement as compared to control in all growth parameter (Table 6). In contrast, C1 provided excellent promotion of growth in all growth parameter. The shoot length of cv. Miya ranged from (6.3cm to 44cm), shoot fresh weights ranged from (2.2g to16g), shoot dray weight ranged from (1.46g to13.3g), root length ranged from (4.03cm to11.3cm), root fresh weight ranged from (2.06g to 5.36 g) and root dray weights ranged from (1.57g to 3.26g) with statically significance deference with control. Table 6 showed that all value in the left hand of the range obtained from seedling treated with B4 and all value in the right hand of the range obtained from seedling treated with C1.

Table 6: The effect *Bacillus* and Cyanobacterial isolates on the growth of tomato cv. Miya seedlings.

Treat	Upper ground growth parameter			Underground growth parameter		
	shoot length in cm	shoot fresh weights in g	shoot dray weights in g	root length in cm	Root fresh weights in g	root dray weights in g
B1	14.6±2.7b *	3.83±0.6b*	2.50±0.2b*	6.00±0.57b*	2.30±0.05b*	1.60±0.05b*
B2	23.0±4.16b*	5.66±0.33b*	2.8±0.08b*	8.0±0.57a*	2.70±0.05b*	1.71±0.00b*
B3	6.33±0.3b*	2.2±0.08b *	1.70±0.1b*	4.03±0.03b*	2.06±0.03b*	1.57±0.05b*
B4	6.33±0.33b*	2.20±0.05b*	1.46±0.1b*	4.03±0.03b*	2.10±0.05b*	1.58±0.06b*
B5	13.66±1.2b*	3.33±0.33b*	2.2±0.05b*	6.00±0.57b*	2.26±0.03b*	1.65±0.00b*
C1	44.0±0.57a**	16.66±0.33a**	13±0.03a**	11.3±0.3a**	5.36±0.18a**	3.26±0.08a**
C2	41.0±0.57a**	12.33±0.33a**	9.63±0.8a*	9.66±0.33a*	3.1±0.1b*	2.11±0.00b*
Cont	3.30±0.35	1.17±0.06	0.76±0.08	2.30±0.05	1.06±0.07	0.48±0.06
LSD at0.05	1.98	1.06	1	1.6	1	0.92

Note: Values are mean± Standard error of three replications, *indicates statistically significant at $p<0.05$ (significant deference with control),** indicates statistically significant at $p<0.01$(highly significant deference with control), Means in each column followed by the same letter are not significantly different at $p<0.05$ according to Fisher's LSD

4.1.8. Efficacy of Bacillus and Cyanobacteria on growth promotion of tomato seedlings

The effect of Bacillus and cyanobacteria isolates on the growth of tomato seedlings was investigated. All five bacillus isolates (B1, B2, B3, B4 and B5) and two cyanobacteria isolates (C1 and C2) show positive effects on the seedling growth of all 3 tomato cultivars Melke salsa**,** Kochero and Miya in all growth parameters including stem and root fresh and dry weights, root length, and stem length, however deferent isolates were showed deference effects on deferent cultivars (Table7).

Table 7: Efficacy of Bacillus and Cyanobacteria on growth promotion of tomato seedlings

Treatment	cv. Melkesalsa		cv. Kochero		cv. Miya	
	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length
B1	54%	66.6%	65%	70%	77%	61.6%
B2	46.23%	61.5%	37%	48.68%	85%	71.2%
B3	57%	68.9%	26%	43.8%	47.8%	42.9%
B4	41%	50%	61%	55%	47.8%	42.9%
B5	62%	62.2%	59%	46.2%	75.84%	61.6%
C1	70%	71.4%	75%	76%	92.5%	79%
C2	64.53%	61.2%	69%	72.3%	91.9%	76.1%
Control	-	-	-	-	-	-

Table7 showed that the growth promotion Efficacy of Bacillus and Cyanobacteria on tomato seedlings were varies from cultivar to cultivar. All Bacillus isolates and Cyanobacterial isolates were showed growth enhancement in all growth parameter of tomato cv. Melke salsa seedlings. In contrast, C1 provided excellent promotion of growth in all growth parameter. The shoot length and root length of cv. Melkesalsa seedlings treated with C1 were showed the highest efficacy (70% and71.4%) respectively. Therefore the effect of C1 on growth promotion of tomato cv. Melkesalsa seedlings was higher than the other treatments and efficacy order of the isolates from higher to lower was C1 followed byC2, B5, B3, B1, B2 and B4.

All Bacillus isolates and Cyanobacterial isolates were showed growth enhancement in all growth parameter of tomato cv. Kochero seedlings. In contrast, C1 provided excellent promotion of growth in all growth parameter. The shoot length and root length of cv. Kochero seedlings treated with C1 were showed the highest efficacy (75% and76%) respectively. There for the effect of C1 on growth promotion of tomato cv. Kochero seedlings was higher than the other treatments and efficacy order of the isolates from higher to lower was C1 followed byC2, B1, B4, B5, B2and B3.

All Bacillus isolates and Cyanobacterial isolates were showed growth enhancement in all growth parameter of tomato cv. Miya seedlings. In contrast, C1 provided excellent promotion of growth in all growth parameter. The shoot length and root length of cv. Miya seedlings treated with C1 were showed the highest efficacy (92.5% and79%) respectively. Miya seedlings treated with C1 were higher than the other treatments and efficacy order of the isolates from higher to lower were C1 followed byC2, B2, B1, B5, B3and B4.

4.1.9. The effect of Bacillus and Cyanobacteria on the growth of Pepper seedlings

Data presented in Table (8 and 9) reveal that all the five Bacillus and tow cyanobacteria isolates caused significant increasing in upper ground and underground growth parameter of Pepper cv. Endiam 42 and cv. Markofana compared with control treatment. The increments of the growth parameters were vary from isolate to isolate and from cultivar to cultivar.

Pepper cv. Endiam42 seedlings were treated with Bacillus isolates and Cyanobacteria isolates, After 45 days of transplantation seedlings were uprooted and their vegetative parameters including stem and root fresh and dray weight, root and stem length were measured. It was observed that treated seedlings showed increase in all growth parameter as compared to non-treated control.

It was observed that treated seedlings of Pepper cv. Endiam42 seedlings treated with C2 showed highest shoot length (20.3±0.66), shoot fresh weight (2.18±0.14), shoot dray weights (2.00±0.11), root length (10.0±0.57), root fresh weight (1.63±0.08) and root dray weight (1.2±0.03) as compared as control and other treatment and the minimum values were recorded on

the seedlings treated with B5 in all growth parameters shoot length(9.90±0.64), shoot fresh weight (0.80±0.02), shoot dray weight (0.72±0.03), root length(6.00±0.57), root fresh weight(0.56±0.07) and root dray weight(0.37±0.05) with statically significance at p<0.5 (Table8). Table 8 showed that among Bacillus species B4 show the maximum shoot length, shoot fresh weghite,shoot dray weghite, root length, root fresh weghit and root dray weghite in the seedlings of Pepper cv. Endiam42.

Table 8: The effect of bacillus and cyanobacteria on the growth of Pepper cv. Endiam42 seedlings

Treatment	Upper ground growth parameter			Underground growth parameter		
	shoot length in cm	shoot fresh weights in g	shoot dray weights in g	root length in cm	Root fresh weights in g	root dray weights in g
B1	9.41±0.36b*	1.24±0.02a*	0.93±0.06b*	8.50±0.76a*	0.66±0.03b*	0.49±0.04b*
B2	12.0±0.57a*	1.50±0.25a*	1.26±0.27a*	6.44±0.30b*	0.84±0.08b*	0.67±0.04b*
B3	13.06±1.1a*	1.63±0.20a*	1.09±0.19a*	8.66±0.33a*	0.69±0.12b*	0.51±0.12b*
B4	15.0±1.15a*	1.93±0.12a**	1.65±0.12a*	9.00±0.57a*	1.49±0.21a**	0.92±0.08a*
B5	9.90±0.64b*	0.80±0.02b*	0.72±0.03b*	6.00±0.57b*	0.56±0.07b*	0.37±0.05b*
C1	19.83±0.9a**	1.85±0.18a*	1.70±0.23a*	10.0±0.16a**	1.25±0.20b*	0.95±0.18b*
C2	20.3±0.66a**	2.18±0.14a**	2.00±0.11a**	10.0±0.57a**	1.63±0.08a**	1.2±0.03a**
Cont	3.91±0.30	0.25±0.02	0.16±0.03	3.33±0.33*	0.12±0.02	0.08±0.01
LSD at0.05	1.2	0.7	0.4	1	0.4	0.2

Note: Values are mean± Standard error of three replications, *indicates statistically significant at p<0.05 (significant deference with control),** indicates statistically significant at p<0.01(highly significant deference with control),ns indicates not statistically significant, Means in each column followed by the same letter are not significantly different at p<0.05 according to Fisher's LSD

Pepper cv. Markofana seedlings were treated with Bacillus isolates and Cyanobacteria isolates, After 45 days of transplantation seedlings were uprooted and their vegetative parameters including stem and root fresh and dray weight, root and stem length were measured. It was observed that treated seedlings showed increase in all growth parameter as compared to non-treated control (Table 9). ANOVA analysis showed significant effect of treatment on vegetative parameters of the plant. The maximum values were recorded on the seedling treated with B4 shoot length(19.0±1.15cm),shoot fresh weight(2.0±0.40 gm), shoot dray weights (1.83±0.12gm),

root length (8.86±0.46), root fresh weight (1.51±0.16) and root dray weight(1.10±0.05) and the minimum values were recorded on seedling treated with B5 shoot length (11.4±0.21), shoot fresh weight (0.95±0.02), shoot dray weights (0.66±0.03), root length (6.30±0.17), root fresh weight (0.40±0.10) and root dray weight(0.25±0.07) which was significantly greater than control and the result was statistically significant at $p<0.05$.

Table 9: the effect of bacillus and cyanobacteria on the growth of Pepper cv. Markofana seedlings

Treatment	Upper ground growth parameter			Underground growth parameter		
	shoot length in cm	shoot fresh weights in g	shoot dray weights in g	root length in cm	Root fresh weights in g	root dray weights in g
B1	14.3±0.6b *	1.95±0.10b*	1.39±0.2b*	5.86±0.13b*	0.86±0.13 b*	0.62±0.09b*
B2	13.9±0.6b*	1.93±0.06b*	1.60±0.05b*	8.33±0.66 a*	1.06±0.66b*	0.79±0.08b*
B3	15.0±1.15b*	2.0±0.15a**	1.86±0.1a**	8.55±0.60 a*	1.36±0.12 b*	1.06±0.06 a*
B4	19.0±1.15a**	2.0±0.40a**	1.83±0.12a**	8.86±0.46a**	1.51±0.16a**	1.10±0.05a**
B5	11.4±0.21b*	0.95±0.02b*	0.66±0.03b*	6.30±0.17b*	0.40±0.10b*	0.25±0.07b*
C1	17.1±1.24 a*	1.90±0.05a*	1.54±0.12 b*	10.0±0.57a**	1.40±0.20a*	1.17±0.15a**
C2	17.0±0.57a*	1.9±0.08a*	1.66±0.08 b*	9.53±0.74a**	1.43±0.14 a*	1.13±0.06a**
Cont	3.50±0.28	0.22±0.03	0.08±0.01	2.67±0.19	0.09±00	0.06±0.01
LSD at0.05	1.97	1.2	0.89	1.43	0.5	0.5

Note: Values are mean± Standard error of three replications, *indicates statistically significant at $p<0.05$ (significant deference with control), ** indicates statistically significant at $p<0.01$(highly significant deference with control), Means in each column followed by the same letter are not significantly different at $p<0.05$ according to Fisher's LSD.

4.1.10. Efficacy of Bacillus and Cyanobacteria on growth promotion of Pepper seedlings

In pot experiment of Pepper plant, comparison of control and treatment plants with one way ANOVA showed that treatment groups have a significant difference in shoot length, shoot fresh and dray weight, root length as well as fresh and dry weight of root as compared with control according to Fisher's LSD at 95%. Pepper Cv. Endiam42 seedlings treated with Bacillus and Cyanobacteria showed growth enhancement as compared as control in all growth parameter. In contrast, C1 provided excellent promotion of growth in all growth parameter. The shoot length and root length of cv. Endiam42 treated with C1 were showed the highest efficacy (80.73%,

66.72%) respectively than other treatment. In opposite to this the shoot length and root length of cv. Endiam42 treated with B1 was showed lower efficacy. There for the effect of C2 on growth promotion of Pepper cv. Endiam42 seedlings was higher than the other treatments and efficacy order of the isolates from higher to lower was C2 followed byC1, B4, B3, B2, B1and B5.

Table: 10 Efficacy of Bacillus and Cyanobacteria on growth promotion of Pepper seedlings

Treatment	cv. Endiam 42		cv. Markofana	
	Shoot length	Root length	Shoot length	Root length
B1	58.44%	60.82%	75.52%	54.43%
B2	67.41%	48.29%	74.82%	67.94%
B3	70%	61.54%	76.66%	68.77%
B4	73.93%	63%	81.57%	73.86%
B5	60.50%	44.5%	69.29%	57.61%
C1	80.28%	66.7%	79.53%	73.3%
C2	80.73%	66.72%	79%	71.98%
Control	-	-	-	-

The above table (Table 10) showed that Pepper Cv. Markofana seedlings treated with Bacillus and Cyanobacteria showed growth enhancement as compared as control in all growth parameter. In contrast, B4 provided excellent promotion of growth in all growth parameter. The shoot length and root length of cv. Markofana treated with B4 were showed the highest efficacy (81.5%, 73.86%) respectively than other treatment. In opposite to this the shoot length and root length of cv. Markofana treated with B5 was showed lower efficacy. There for the effect of B4 on growth promotion of Pepper cv. Markofana seedlings was higher than the other treatments.

Figure4 uprooted pepper seedling

4.1.11. The effect of Bacillus and Cyanobacteria on the growth of Rice seedlings

In pot experiment of Rice plant, comparison of control and treatment plants with one way ANOVA showed that treatment groups have a significant difference in shoot length, shoot fresh and dray weight, root length as well as fresh and dry weight of root as compared with control. However, effect of Bacillus and Cyanobacterial isolates are not the same in different cultivars of Rice plants.

It was observed that among the five Bacillus isolates B3 showed highest shoot length, shoot fresh weight, shoot dray weight, root length, root fresh weight and root dray weight of Cv. X-jegna as compared as other bacillus isolates with statistically significance at 0.05 level of significance, whereas B5 was not showed statically significant effect on vegetative characters of studied plants of Cv. X-jegna as compared as control (Table 11). Also it was showed that among all treatments C2 was showed highest mean value in all growth parameter.

Table 11: The effect of Bacillus and Cyanobacteria on the growth of Rice Cv. X-jegna seedlings

Treatment	Upper ground growth parameter			Underground growth parameter		
	shoot length in cm	shoot fresh weights in g	shoot dray weights in g	root length in cm	Root fresh weights in g	root dray weights in g
B1	46.0±1.15b*	6.00±0.11b*	4.21±0.12b*	21.33±0.3b*	2.8±0.06b*	1.80±00b*
B2	40.6±0.66b*	4.9±0.05b*	3.46±0.13b*	17.3±0.32c*	2.53±0.01b*	1.50±0.05c*
B3	52.3±1.20a*	7.10±0.05a*	5.1±0.05a**	24.6±0.33b*	3.13±0.03a*	1.95±0.02b*
B4	50.3±0.33a*	6.23±0.28b*	4.08±0.08b*	21.6±0.88b*	2.89±0.05b*	1.92±0.02b*
B5	38.0±2.30ns	4.00±0.11ns	2.7±0.04ns	14.6±0.33ns	1.0±0.10ns	0.54±0.11ns
C1	57.0±0.57a**	7.50±0.05a**	5.56±0.08a**	27.0±0.57a*	4.50±0.05a*	2.79±0.05a*
C2	58.0±0.57a**	7.53±0.03a**	5.56±0.08a**	28.3±0.66a**	4.80±0.05a**	3.06±0.03a**
Cont	36.0±4.00	3.65±0.05	2.65±0.05	14.5±0.50	0.95±0.05	0.43±0.06
LSD at0.05	3.21	2.19	3	3.12	0.98	1.2

Note: Values are mean± Standard error of three replications, *indicates statistically significant at $p<0.05$ (significant deference with control), ** indicates statistically significant at $p<0.01$(highly significant deference with control), ns indicates not statistically significant, Means in each column followed by the same letter are not significantly different at $p<0.05$ according to Fisher's LSD.

It was observed that among the five Bacillus isolates B3 showed higher mean value on most vegetative characters of Cv. Edget as compared as other Bacillus isolates, where as B4 was not showed statically significant effect on vegetative characters of studied plants of Cv. Edget as compared as control. Also it was showed that among all treatments C2 was showed higher mean values on most vegetative characters of Cv. Edget as compared as other treatments. And also it was showed that among bacillus isolates B4 showed a little effect but statically it was not significant compared as control group or untreated seedlings of rice cv. Edget. In addition to this all treatment except B4 showed increase number of tiller or twigs.

Table 12: The effect of Bacillus and Cyanobacteria on the growth of Rice Cv. Edget seedlings

Treatment	Upper ground growth parameter			Underground growth parameter		
	shoot length in cm	shoot fresh weights in g	shoot dray weights in g	root length in cm	Root fresh weights in g	root dray weights in g
B1	40.0±1.00 b*	5.33±0.24b*	3.6±0.11b*	17.3±0.66 c*	1.96±0.03b*	1.40±0.11b*
B2	48.3±1.2 b*	5.73±0.14b*	3.95±0.02b*	21.3±0.33 b*	2.66±0.14b*	1.73±0.03b*
B3	49.6±0.33b*	6.32±0.33b*	4.46±0.37b*	21.3±0.66 b*	2.86±0.03 b*	1.76±0.03b*
B4	34.0±1.15ns	4.33±0.08ns	2.10±0.05ns	14.6±0.33ns	1.60±0.05ns	0.9±0.05ns
B5	41.0±0.57b*	5.40±0.11b*	3.30±0.15b*	16.6±0.33c*	1.90±0.05 c*	0.90±00ns
C1	58.3±1.45a**	7.53±0.08a**	6.10±0.06a**	28.6±0.33a*	4.10±0.20 a*	2.69±0.04a*
C2	63.3±0.33a**	7.93±0.03a**	6.41±0.04a**	31.0±1.15a**	4.6±0.03a**	3.00±0.05a**
Cont	31.6±0.88	4.0±0.10	2.03±0.04	13.6±0.88	1.43±0.03	0.83±0.12
LSD at0.05	3.8	3.12	0.7	0.57	0.64	0.54

Note: Values are mean± Standard error of three replications, *indicates statistically significant at $p<0.05$ (significant deference with control), ** indicates statistically significant at $p<0.01$(highly significant deference with control), ns indicates not statistically significant, Means in each column followed by the same letter are not significantly different at $p<0.05$ according to Fisher's LSD.

It was observed that among the five Bacillus isolates B4 showed higher mean values on most vegetative characters of Cv. Getachew as compared as other bacillus isolates, whereas B1 was not showed statically significant effect on vegetative characters of studied plants of Cv. Getachew as compared as control (Table 13). Also it was showed that among all treatments C2 was showed higher mean value as compared to other treatment. And also it was showed that

among bacillus isolates B1 was not showed any effect compared as control group or untreated seedlings of rice cv. Getachew. In addition to this all treatment except B1 showed increase number of tiller or twigs.

Table 13: The effect of Bacillus and Cyanobacteria on the growth of Rice Cv. Getachew seedlings

Treatment	Upper ground growth parameter			Underground growth parameter		
	shoot length in cm	shoot fresh weights in g	shoot dray weights in g	root length in cm	Root fresh weights in g	root dray weights in g
B1	38.66±0.3ns	4.8±0.05ns	3.00±0.06ns	16.6±0.33ns	1.80±0.05ns	1.07±0.03ns
B2	50.0±0.57b*	5.60±0.57b*	3.73±0.08b*	24.0±0.57b*	3.00±0.05b*	1.96±0.03b*
B3	53.3±0.66b*	5.80±0.05b*	3.76±0.06b*	25.0±0.57b*	3.13±0.08b*	2.10±0.05b*
B4	54.3±0.88b*	5.50±0.05b*	3.73±0.08b*	25.0±0.57b*	3.03±0.03b*	2.16±0.08b*
B5	43.0±0.57c*	5.13±0.06b*	3.03±0.04ns	20.0±0.57c*	2.10±0.05c*	1.48±0.06c*
C1	63.33±4.6a**	7.66±0.33a*	5.22±0.23a*	29.0±1.15a*	5.2±0.16a*	4.06±0.17a**
C2	63.3±0.66a**	9.40±0.2a**	6.20±0.15a**	32.0±0.57a**	6.0±0.11a**	4.73±0.06a**
Cont	38.6±0.88	4.8±0.10	2.89±0.01	16.3±0.33	1.75±0.02	1.00±0.00
LSD at0.05	4.01	3.1	0.9	4.06	3.1	0.98

Note: Values are mean± Standard error of three replications, *indicates statistically significant at $p<0.05$ (significant deference with control), ** indicates statistically significant at $p<0.01$(highly significant deference with control), ns indicates not statistically significant, Means in each column followed by the same letter are not significantly different at $p<0.05$ according to Fisher's LSD.

4.1.12. Efficacy of Bacillus and Cyanobacteria on growth promotion of Rice seedlings

All five bacillus and tow cyanobacterial isolates were showed that improvement of growth parameter in rice seedlings .However there was difference Efficacy among the isolates from cultivar to cultivar.

Rice cv. X-jigna seedlings treated with Bacillus and Cyanobacteria showed growth enhancement as compared as control in all growth parameter. In contrast, C2 provided excellent promotion of growth in all growth parameter. The shoot length and root length of cv. X-jigna treated with C2 was showed the highest efficacy (37.93%, 50.53 %) respectively than other treatment. In opposite to this the shoot length and root length of cv. X-jigna treated with B5 was showed lower

efficacy. There for the effect of C2 on growth promotion of Rice cv. X-jigna seedlings was higher than the other treatments.

Table1 14: Efficacy of Bacillus and Cyanobacteria on growth promotion of rice seedlings

Treatment	cv. X-jigna		cv. Edget		cv. Getachew	
	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length
B1	21.73%	32.02%	21%	21.38%	0.15%	1.80%
B2	11.33%	16.18%	34%	36.18%	22.8%	32.08%
B3	31.16%	41.05%	36.29%	36.18%	27.57%	34.8%
B4	28.42%	32.03%	7.05%	6.84%	28.91%	34.8%
B5	5.26%	0.68%	22.02%	18.07%	10.23%	18.5%
C1	36.84%	46.29%	45.79%	52.44%	39.04%	78.27%
C2	37.93%	50.53%	50.07%	52.9%	39.02%	61.57%
Control						

Rice cv. Edget seedlings treated with Bacillus and Cyanobacteria showed growth enhancement as compared as control in all growth parameter. In contrast, C2 provided excellent promotion of growth in all growth parameter. The shoot length and root length of cv. Edget treated with C2 was showed the highest efficacy (50.07%, 52.9 %) respectively than other treatment. In opposite to this the shoot length and root length of cv. Edget treated with B4 was showed lower efficacy. There for the effect of C2 on growth promotion of Rice cv. Edget seedlings was higher than the other treatments.

Rice cv. Getachew seedlings treated with Bacillus and Cyanobacteria showed growth enhancement as compared as control in all growth parameter. In contrast, C1 provided excellent promotion of growth in all growth parameter. The shoot length and root length of cv. Getachew treated with C1 was showed the highest efficacy (39.04%, 78.27 %) respectively than other treatment. In contrary to this the shoot length and root length of cv. Getachew treated with B1 was showed lower efficacy. There for the effect of C1 on growth promotion of Rice cv. Getachew seedlings was higher than the other treatments.

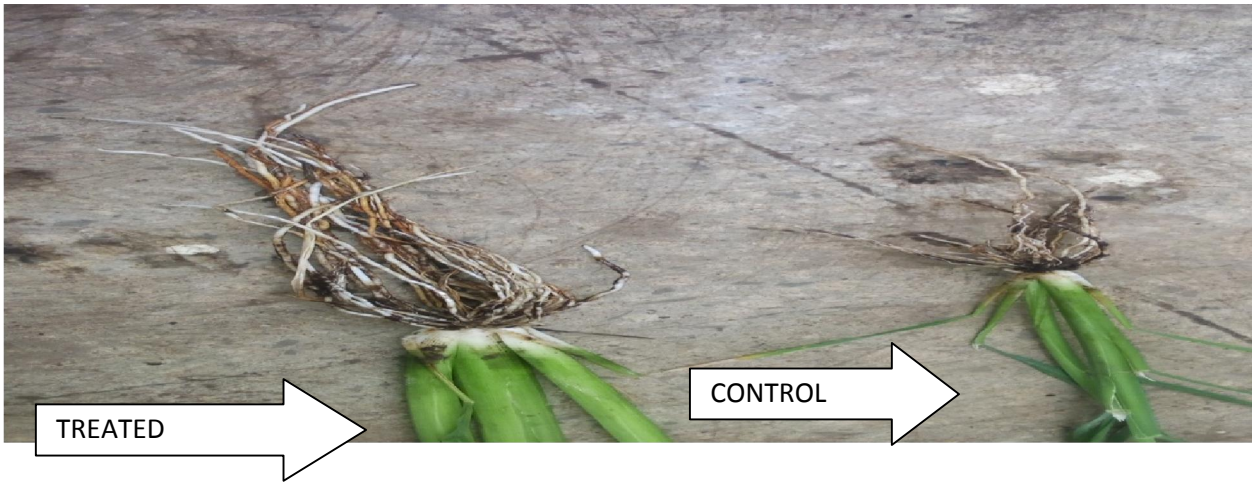

Figure 5Uprooted Rice seedlings

Figure 6 Rice seedlings on pot experiment

4.2. DISCUSSION

Bacillus species are physiologically diverse and this can be grouped together based on similarities in morphological, physiological and biochemical characters. Many studies have suggested that the strains of the genus *Bacillus* are more heterogeneous than most other bacterial genera (Priest *et al*., 1988). In Bergey's manual of systemic Bacteriology there are six genera of endospore forming bacteria featured. *Bacillus* species are distinguished from the other endospore forming bacteria on the basis of being a strict or facultative aerobe, rod shaped and usually Catalase positive. Here 100% of the identified isolates are rod shaped and Catalase positive.

In the present study 5 *Bacillus* species were isolated from sediments of Lake Tana and 2 cyanobacterial species were isolated from the samples collected around Gondar showed significant increase to all growth parameter of treated all 3 tomato cultivars seedlings compared with control or untreated seedlings, but varied their efficiency in deferent cultivars (Table 6,7and 8). The results clearly demonstrate that differences in the PGPR properties of the individual isolates made wide-ranging their effectiveness in deferent cultivars. This result was in agreement with the report of (Sivasakthi *et al,* 2014, Vejan *et al.*, 2016). They have suggested that different effectiveness of deferent isolates were due to the variety of plant growth enhancing mechanisms. The mode of action of PGPR that promotes plant growth includes (i) abiotic stress tolerance in plants; (ii) nutrient fixation for easy uptake by plant; (iii) plant growth regulators; (iv) the production of siderophores; (v) the production of volatile organic compounds; and (vi) the production of protection enzyme such as chitinase, glucanase, and ACC-deaminase for the prevention of plant diseases . However, the mode of action of different PGPR varies depending on the type of host plants (Sivasakthi *et al.,* 2014, Vejan *et al.*, 2016)

This study revealed that significant improvement was made on seedling growth due to Bacillus and Cyanobacterial inoculation. The treated seedling showed that the highest root length and root fresh and dray weights as compared to control or untreated seedling .This result agree with the study done by (Mia *et al.*, 2014) on Effects of rhizobia and plant growth promoting bacteria inoculation on germination and seedling vigor of lowland rice. It has been suggested that Induction of longer roots with increased number of root hairs and root laterals is a growth response attri- buted to IAA production by PGPR, therefore the Bacillus isolated from Lake Tana and cyanobacteria that used in this study enhance plant growth could be through IAA

production.

The present experiment revealed that cyanobacterial inoculation was an effective treatment for improving the all parameters measured shoot length, shoot fresh and dray weights, root length, root fresh and dray weights in all tomato cultivar, pepper cultivar and rice cultivar. The results clearly demonstrated that both isolated Cyanobacteria C1 and C2 having one or more mechanisms of plant growth enhancing traits. Early studies by (Kim, 2008) have shown that treated seedlings by cyanobacteria significantly inhibited the growth of *Candida albicans* and *Sclerotinia sclerotiorum* and produce exopolysaccharides that can function as energy sources for fungi and produce plant growth regulators, which are abscisic acid, ethylene, jasmonic acid, auxin, and cytokinin-like substances, the cytokinin isopentenyl adenine. These substances can influence fungal growth which leads wilts of vegetable and crop seedlings and the work of (Mazhar and Hasnain, 2011) was showed that Cyanobacterial strains may protect plants from phyto pathogens due to hydrogen cyanide production and also other previous work showed that the effect of cyanobacteria on the growth of seedlings was came from the phyto hormone production ability of cyanobacteria(Mia *et al.*, 2014). Induction of longer roots with increased number of root hairs and root laterals is a growth response attri- buted to IAA production by Cyanobacteria. . Nostoc and Anabaena are the prokaryotic organisms and phototropic in nature. They play an important role in enriching paddy field soil by fixing atmospheric nitrogen and supply vitamin B complex and growth promoting substances which make the plant to grow vigorously(Youssef and Eissa, 2014).

In the present study 5 *Bacillus* species were isolated from sediments of Lake from this one isolate was *Bacillus thuringiensis* and has showed a positive effect on tomato seedlings. The result of present study was agreed to Early studies by (Aiuchi *et al*, 2016) have shown that *B. thuringiensis* strains promoted tomato shoot and root elongation compared to the untreated control . *Bacillus thuringiensis* has been used as an effective bio insecticide because it produces the proteins Cry and Cyt, which are highly toxic to insects and produces several compounds, such as antimicrobial substances that include exo toxins, antibiotics, degrading enzymes, bacteriocins, and a signal molecule in the bacterial quorum-sensing system.

Another *Bacillus* species in the present study isolated from sediments of Lake Tana was *B.licheniformis* and has showed the positive effect on plant growth. In this study the seedling of

tomato, pepper and rice seedling treated with *B.licheniformis* showed that increase in all parameter compared as control untreated seedling. This result agreed to the early study done by*(*Ajilogba *et al* 2013) have shown that *B.licheniformis* increase in leaf, stem and root growth of red pepper and tomato seedlings. The growth promotion of the tomato, pepper and rice seedlings by *B.licheniformis* possibly attributed to the production of antifungal β-glucanase, siderophere and auxins and also involved in phosphate solubilization*(*Ajilogba *et al,*. 2013).

5. CONCLUSION AND RECOMMENDATIONS

5.1. CONCLUSION

The microorganisms isolated from sediment of Lake Tana were characterized and identified as *Bacillus* species. All 5 *Bacillus* isolates *B.sphaericus, B.licheniformis, B.thuringiensis, B.pumilus and B.thermophiles* isolated from sediment of Lake Tana and the microorganisms isolated from Gondar area were characterized and identified as *Cyanobacteria* species, *Anabaena* and *Nostoc* showed a positive effects on the growth of pepper, rice and tomato seedlings .Especially both Cyanobacteria isolates showed great positive effects on the treated seedling without cultivar selection. Therefore, this group is possibly used for organic fertilizer or biofertilizer production. Generally the potential of these microorganisms to produce a healthy and vigor seedling is great and they have abilities to avoid crop and vegetable lose that leads to a new mechanisms choice for the control of pesticide resistant phytopatogen and escape the early die of pepper, rice and tomato seedlings.

In general the current study revealed that the use of Bacillus isolates isolated from sediment of Lake Tana and Cyanobacteria isolated from Gondar area were showed the improvement of all growth parameter which are shoot length, fresh and dray weights and root length, fresh and dray weights as compared as control.

5.2. RECOMMENDATIONS

Based on these findings, the following recommendations are given:

PGPR tests are recommended to be done on fields how much increasing the yield of the crop and there should be demonstrated to the end users who are having Agricultural importance.

Molecular characterization of the strains is necessary to make more use of these isolates.

The combination effect of different isolates on growth promotion of pepper, tomato and rice seedlings tests are recommended to be done field plots.

The plant growth promotion mechanisms of these Bacillus and cyanobacteria isolates to be assessed

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ANNEX1
Biochemical tests for Bacillus isolation

Indole Test: One percent tryptophan broth in a test tube was inoculated with bacteria colony. After incubation period of 37°c for 48hours, then one milliliter (1ml) of chloroform was added to the broth. The test tube was shake gently, then 2ml of Kovac's reagent was added and this is also shaken gently and allowed to stand for twenty (20) minutes. The formation of red coloration at the top layer indicated positive and yellow coloration indicates negative.

Catalase Test: This was carried out by putting a drop of hydrogen peroxide on a clean slide. With the edge of another slide, a colony of the organism was pick and allow to be in contact with the hydrogen peroxide. Presence of bubbles indicates positive reaction while absence of bubble indicates negative reaction.

MR-VP Test; Five milliliters (5ml) of MR VP broth was inoculated with the isolated *Bacillus* and incubated for 48-72 hours at 37°c after which, one milliliter (1ml) of the broth was transferred into a small tube. Some small quantity (2or3 drops) of methyl red tests were added. A red color one in the addition of the indicator signified a positive methyl red test while yellow color signified a negative test. To the rest of the broth in the original tube some drops (five) of 4% potassium hydroxide (KOH) were added followed by some (fifteen) drops of 5% naphtol in ethanol. The test tube (sealed with cotton plug) was shaken and place in a sloping position. The development of a red color starting from the liquid-air interface within 1 hour indicated a VP positive test while no color change indicated a VP negative test (Manga and oyeleke, 2008).

Triple Sugar Iron Agar Test (TSI): The medium contains three (3) sugars namely: glucose, lactose and sucrose. The pH indicator is phenol red and detection system for hydrogen sulphide (H_2S) is included. This medium was prepared as agar slope and the test organism will be inoculated by stabbing the medium with the aid of sterilized straight wire loop and the surface of slope was inoculated by streaking and then incubated at 37°C for 24hours, after which observation was made. Gas production was determined by cracking of the medium, formation of H_2S was determined by the blackening of the whole buffer or a streak of ring of blackening at the slant butt junction, glucose fermentation was determined by the yellowing of the butt. The fermentation of lactose or sucrose or both was determined by the yellowing of both the butt and the slant and the motility was determined by observing the line inoculation; sharply defined line of inoculation indicating positive motility (Manga and oyeleke, 2008).

Starch Hydrolysis: *Bacillus* isolates were streaked on starch agar plates and incubated at 37^0C for 24 hrs. After the incubation period iodine solution was poured on the agar and examined for the hydrolysis of starch by production of clear zone around the microbial growth as indicated by development of blue color by starch with iodine.

Gelatine Hydrolysis: This test was carried out by streaking *Bacillus* isolates on gelatin agar plates and incubating at 37^0C for 24 hrs. Following incubation, the plates was flooded with 1ml

of mercuric chloride solution and observed for zone of hydrolysis. A positive result was indicated by the presence of a clear zone surrounding the colony.

Casein Hydrolysis: *Bacillus* isolates were streaked on skimmed milk agar medium and incubated at 37^0C for 24 hrs. Positive results were indicated by the presence of clear zone surrounding the colony.

Urea Hydrolysis: *Bacillus* isolates were inoculated to sterile urea agar slants and incubated at 37^0C for 24 hrs. Observations were made daily to distinguish positive and negative results. Positive results were confirmed by the change in color of the agar slant to pinkish red.

Citrate Utilization Test: Isolates were streaked on Simon's citrate slant agar and incubated at 37^0C for 24 hrs. Positive results were indicated by color changing of media from green to intense blue color.

Resistance towards Sodium Chloride: Nutrient agar was prepared in three batches which were supplemented with 5%, 7% and 10% sodium chloride. The medium was autoclaved and solidified in plates. Agar plates were then divided into sectors with each sector being streaked with isolates. The plates were incubated at 37^0C for 24 hrs and visual observations were made to record the growth of *Bacillus* at the highest concentration of salt.

Determination of Optimum Temperature for Growth: The *Bacillus* isolates were streaked on nutrient agar plates. The plates were incubated at 20°C, 30°C, 35°C, 37, 45°C, and 55°C for 24-48 hrs. The optimum temperature was determined by visual examination of their growth.

ANNEX 2

$BG11_0$ formulation for cyanobacterial isolation

Macro-nutrient solutions:

Macro nutrient solutions were prepared as follow:

40 mg	K2HPO4
75 mg	MgSO4.7H2O
36 mg	CaCl2.2H2O
6 mg	Citric acid
20 mg	Na2CO3
1 mg	Na2EDTA;
6 mg	Ferric ammonium citrate

The above salts were dissolved in 1 litre distilled water, and then 1 ml of the micro-element solution was added.

Micro-nutrient solutions:

Micro nutrient solutions were prepared as follows:

2.86g	H3BO3
1.81g	MnCl2.4H2O
0.222g	ZnSO4.7H2O
0.39g	Na2MoO4.2H2O
0.079g	CuSO4.5H2O
0.0494g	Co (NO3)2.6H2O

Table1: The colony morphology and microscopic result of the isolated bacterial strains

Colony Character	Bacillus species B1	B2	B3	B4	B5
Color	White	White	White	White	White
Form	Circular	Circular	Circular	Irregular	Circular
Elevation	Convex	Convex	Flat	Flat	Flat
Margin	Entire	Entire	Entire	Lobate	Entire
Endospores	+	+	+	+	+
Shape	Rod	Rod	Rod	Rod	Rod

Table 2:- Physiological and Biochemical Characteristics of *Bacillus* Species isolated from Lake Tana sediment samples

Biochemical tests	Bacillus species B1	B2	B3	B4	B5
Gram Rex	+	+	+	+	+
Catalase Test	+	+	+	+	+
Casein Hydrolysis	+	+	+	+	-
Citrate Utilization	-	+	+	+	-
VP test	-	-	+	-	-
MR test	+	-	-	+	+
Indole test	+			-	+
H_2S Production	+	+	-	-	+
Glucose (Acid)	+		-	+	+
Glucose (Gas)	+	-	-	-	+
Urea Hydrolysis	+	-	+	+	-
Starch Hydrolysis	+	-	-	+	-
Gelatin Hydrolysis	+	-	+	+	+
Resistance to 5% NaCl	+	+	-	+	+
Resistance to 7% NaCl	+	+	-	+	-
Resistance to 10% NaCl	+	-	-	-	-

Growth at 20°C	-	-	+		-
Growth at 30°C	+	+	+	+	+
Growth at 37°C	+	+	+	+	+
Growth at 45°C	+	-	-	-	-
Growth at 55°C	+	-	_	-	-
possible specie	*B.thermophiles*	*B.thuringiensis*	*B.sphaericus*	*B.licheniformis*	*B.pumilus*

Table3: The colony morphology of the isolated Cyanobacterial strains

Morphology of the isolate	Cyanobacterial isolates C1	C2
Colonial vs filamentous	Coiled filament	Colonial
Color	greenish	greenish
Margin	Entire	Entire
Form	Un branched filament	Circular
Gram reaction	-	-
Shape	Spherical bed like	Cylindrical
promising species	*Anabaena*	*Nostoc*

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Figure1: Cyanobacteria isolate colony morphology

DECLARATION

First, I declare that this thesis is my work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillments of the requirements for an MSc degree at the University of Gondar and is deposited at University Library to be made available to borrowers under rules of the Library. I seriously declare that this thesis is not submitted to any other institution for the award of any academic degree, diploma, or certificate.

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The work has been done under my supervision

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